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ANALYSIS OF PHYSICOCHEMICAL PROPERTIES OF ELECTROSPUN POLYMER SCAFFOLDS TO BE USED IN TISSUE ENGINEERING

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Abstract - The electrospinning technique was used to develop nanofibers with pure PDLLA and mixed with Spirulina (PDLLA/Sp). The scaffolds were evaluated for morphology, fiber diameter, pore size, contact angle with water, residual solvent content, roughness and autofluorescence. PDLLA fibers showed homogeneous morphology, whereas PDLLA/Sp fibers showed more heterogeneous morphology. The average fiber diameter and average pore size for both matrices were similar. The contact angle and roughness observed in the matrices of PDLLA were higher than those of PDLLA/Sp. The autofluorescence showed greater intensity in the PDLLA/Sp compared with the PDLLA scaffolds, due to the presence of microalga Spirulina in the PDLLA/Sp scaffolds. This new biomaterial using Spirulina can be used to produce funcionalized scaffolds. Analysis suggest that both matrices are good candidates as biomaterial in nanomedicine, focusing on tissue engineering, due to their morphology and diameter of fibers being similar to the natural extracellular matrix of cells and low residual solvent.
Keywords: *Scaffolds, nanotechnology, electrospinning, tissue engineering, Spirulina*

Introduction

Nanotechnology and regenerative medicine are the major innovations of this century and the association of these two innovative areas, through the combination of the use of nanofibers and stem cells are being strongly indicated as candidates for future regenerative therapy of organs and tissues [1,2]. In this work two different nanofiber scaffolds constructed by electrospinning were developed. Both have the poly-D, L-lactic acid (PDLLA) polymer as a constituent, which is biocompatible and biodegradable [3]. However, only one of them has the inclusion of Spirulina (Sp) with known antibacterial, antifungal and anti-inflamatory properties [4,5] (MICHELE). These molds degrade while the regeneration and replacement of a tissue occurs [6]. The nanofibers produced by electrospinning mimic the structure of the extracellular matrix [7].

Therefore, the aim of this study has been to observe the physical and chemical properties that enhance the biocompatibility and degradation of nanofibrous scaffolds to serve as support where mesenchymal stem cells can be cultured in order to develop a dermal substitute for skin regeneration in burn patients.

Experimental

Preparation of polymer solutions

The polymer solution consisting of PDLLA (molecular weight 75,000 to 120,000) (Sigma) was produced at a concentration of 7% (w/w) using 1,1,1,3,3,3-hexafluoride-2-propanol (HFIP) (Sigma). Meanwhile, PDLLA/Spirulina (PDLLA/Sp) solution was prepared at 8% (w/w) of PDLLA in HFIP, plus 2% of the biomass of Spirulina. The microalga was obtained and prepared according to the norms established by this group [5].

Production of nanofibers by electrospinning technique

The construction of nanofiber biomaterials was performed by the electrospinning method. The voltage used for the PDLLA solution was 20 kV, needle inner diameter of 0.45 mm and flow of 1.88 mL/h. The voltage used for the PDLLA/Sp solution was 15 kV, needle inner diameter 0.60 mm and a flow rate of 2 mL/h. The distance between the needle and the collector for both solutions was 15 cm. The group has required a patent concerning the use of Spirulina to produce scaffolds by electrospinning technique for tissue engineering proposals.

Morphology and diameter of the fibers

The morphology of the nanofiber matrices was evaluated by scanning electron microscopy (SEM) model JOEL - JSM 6060, with acceleration voltage of 10 kV. The diameter of the fibers was evaluated by ImageJ software.

Pore size

The pore size was evaluated in SEM images of three samples of each type of scaffold, using the software ImageJ.

Contact angle

The contact angle analysis was performed in five samples of each type of scaffold. Using the software Surftens 3.0, three measurements were made of each photo.

Content of residual solvent

The residual solvent content was evaluated by thermogravimetric analysis (TGA). The samples were submitted to 25°C for 5 minutes in a nitrogen atmosphere, followed by a heating program of 20°C/min in a temperature range between 25 to 750ºC and then 70°C/min up to 800°C. The

equipment used was an SDT Q600 (TA Instruments). Graphic analysis was accomplished in the TA Universal Analysis software.

Roughness

The roughness (Ra) was assessed using photographs of atomic force microscopy (AFM) of two different regions of three different matrices for each sample type. The equipment used was Nano Scope ® Scanning Probe Microscope Controller - Digital Instruments.

Analysis of autofluorescence

We analyzed three different samples of each type of matrix. In each sample two different regions were photographed. For the analysis of both matrices the same parameters were used: lasers 403, 473 and 559 nm, with 30% intensity. In addition, scans were also performed in various wavelengths (λ) for determining in which λ the highest fluorescence emission occurs.

Results and Discussion

Morphology, fiber diameter and pore size

The fibers made of PDLLA showed homogeneous morphology without the presence of beads (Fig. 1A) and with an average diameter of 276±65.9 (163-581) nm. The average size of the pores was between 2.569±1.279 (0.525 to 6.312) micrometers. The fibers made of PDLLA/Sp showed more heterogeneous morphology (Fig. 1B). These fibers have an average diameter of 263±82 (91-576) nm and the pore size observed was 2.395±1.047 (0.327 to 5.449) micrometers.

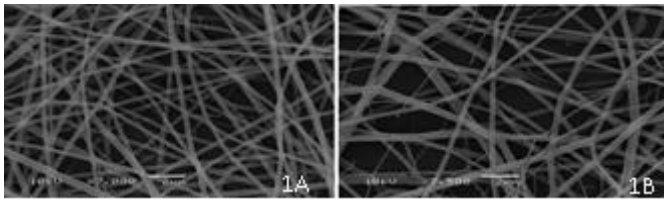

Figure 1: SEM photographs: A) PDLLA with a magnification of 7,000x. B) PDLLA/Sp with a magnification of 7,500x.

As can be seen in Figure 2, the fibers have a very wide variation in diameter range. This property gives an advantage to the technique as it closely mimics the collagen fibers in normal extracellular matrices, where the diameter varies from 50 to 500 nm [8]. Because the pores are interconnected, they allow the migration of cells that fill the matrix structure. They also allow the entrance of nutrients and growth factors and the removal of metabolic products of the cells [9].

Contact angle

The contact angle in the PDLLA samples ranged between 94.1° and 127°, with an average of 121.41°±0.26° (Fig. 3A), while in the PDLLA/Sp matrices the values were between 70.4° and 127°, with an average value of 111.47°±0.25° (Fig. 3B). Both scaffolds are considered to have hydrophobic contact angles.

This measurement is an important factor to be observed because it reflects the hydrophilicity of the matrix and consequently the matrix-cell interaction.

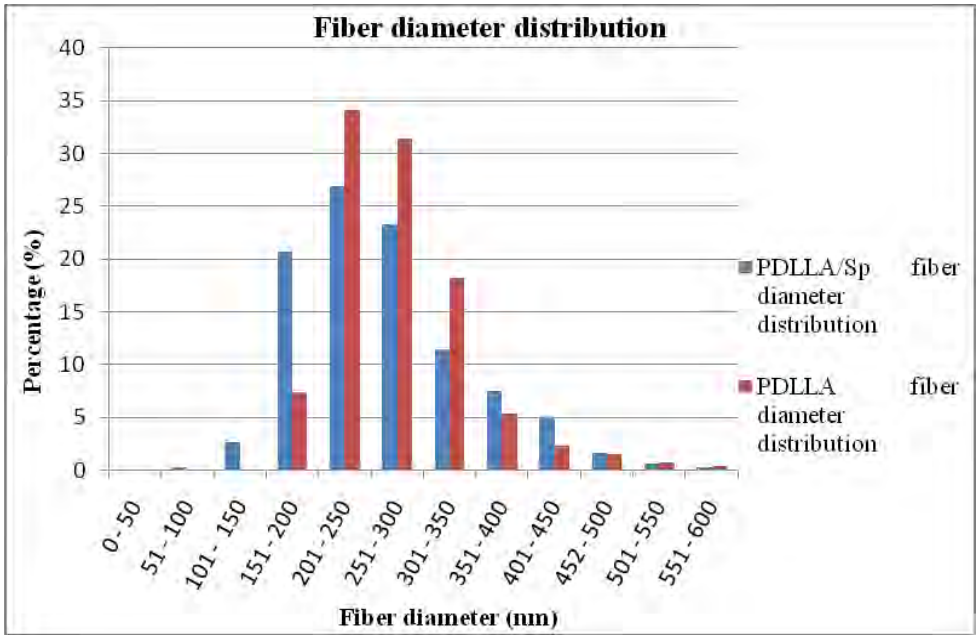

Figure 2: Demonstration of the distribution of fiber diameters from both matrices used in the study.

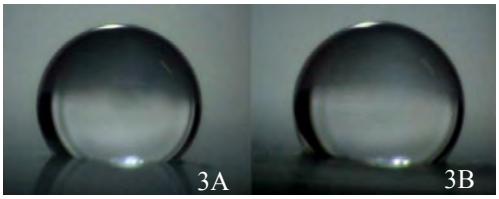

Figure 3: Photographs of the contact angle with water test. A) PDLLA. B) PDLLA/Sp.

Content of residual solvent

The use of organic solvents, which are mostly toxic to cells, led to the verification of the complete absence of solvents in the fabricated matrix.

Three separate groups were evaluated: (1) Spirulina biomass (Fig. 4A), (2) PDLLA matrix (Fig. 4B), and (3) PDLLA/Sp matrix (Fig. 4C).

Mass loss at temperatures below 100°C normally refers to the volatilization of solvents present in the sample, being either water or an organic solvent. No organic solvent was used to analyze the biomass of Spirulin. The scaffolds had been previously treated with HFIP for the production of polymer solution and subsequent electrospinning. By comparing the graphs obtained, it is assumed

that the solvent HFIP is absent or, if present, it is in very low concentrations, as the biomass was not brought into contact with organic solvents and it also has the same sign. Therefore, it is believed that the first weight loss shown in the baseline refers to water, which is very common in plant cells and that can also be present in the scaffolds as moisture.

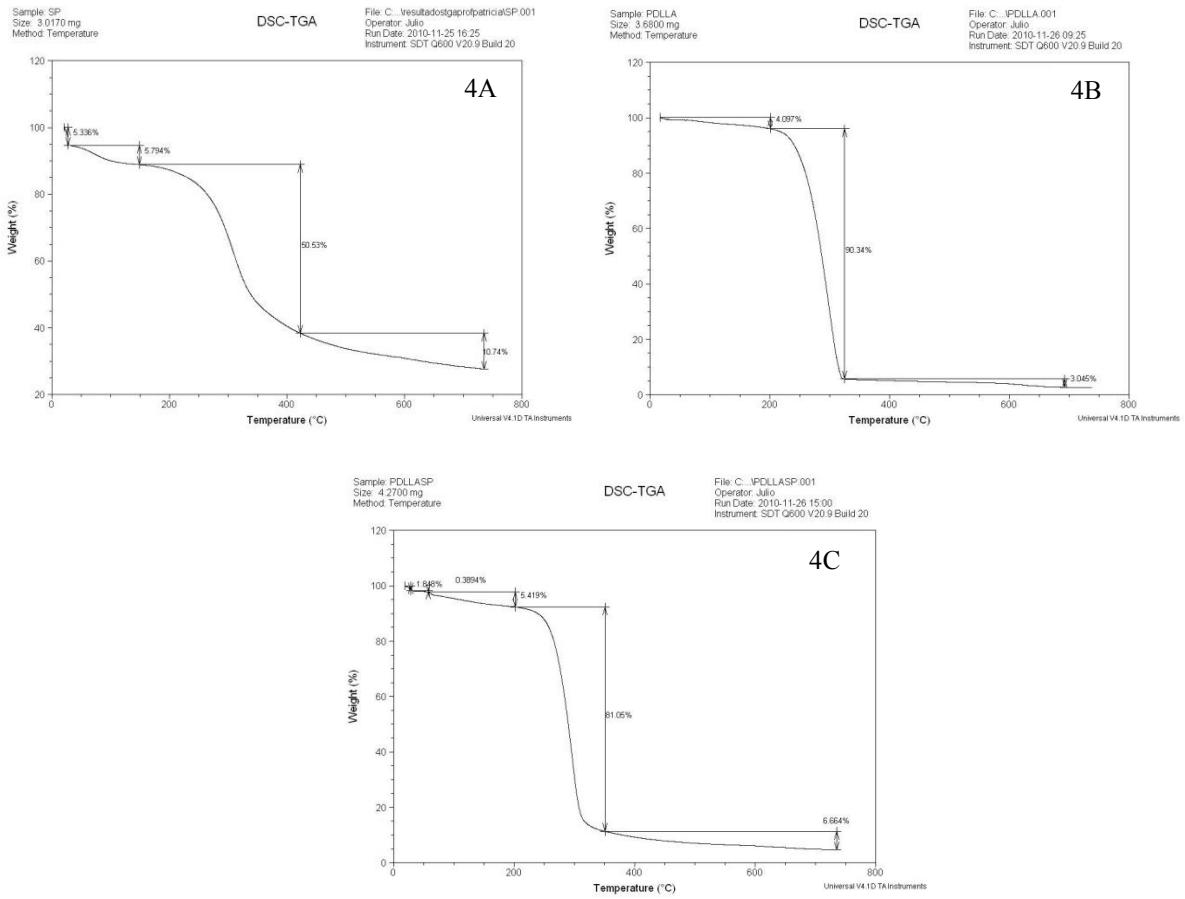

Figure 4: A) TGA graph of the Spirulina biomass; B) TGA graph PDLLA matrix; C) TGA graph PDLLA/Sp matrix.

Roughness

This parameter directly influences the adhesion, proliferation and cell vitality [10]. The average roughness (Ra) of the PDLLA matrix obtained was 413.1 ± 68.9 (289.9 to 493.8) nm (Fig. 5A). On the other hand, the scaffolds made of PDLLA/Sp had an average roughness of 409.9 ± 122.6 (261.2 to 589.9) nm (Fig. 5B).

Analysis of autofluorescence

The scan performed at various wavelengths (λ) for determining which of these has the largest emission of fluorescence by the matrices showed that both have the highest fluorescence emission in the same energy range, between 565 and 575 nm.

With regard to the intensity of autofluorescence, the PDLLA/Sp matrix showed greater intensity compared to the PDLLA matrix, which confirms the incorporation of Spirulina in its constitution.

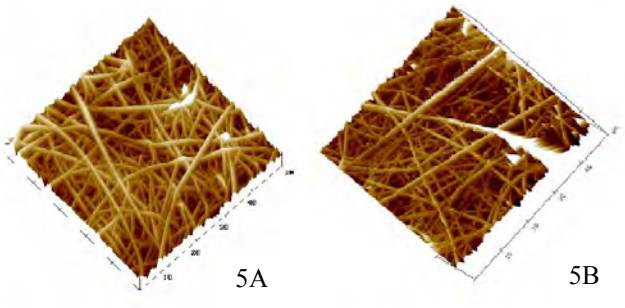

Figure 5: A) AFM image of the PDLLA matrix, B) AFM image of the array of PDLLA/Sp.

Conclusions

These analyses show that these matrices are very promising candidates for biomaterial in nanomedicine with a focus on tissue engineering for presenting morphology and fiber diameter similar to the natural extracellular matrix of cells.

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